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Determination of Asperosaponin VI in Dog Plasma by High-Performance Liquid Chromatography–tandem Mass Spectrometry and its Application to a Pilot Pharmacokinetic Study

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A sensitive and rapid liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed and validated for the determination of asperosaponin VI in beagle dog plasma using glycyrrhizic acid as the internal standard (I.S.). Plasma samples were simply pretreated with methanol for deproteinization. Chromatographic separation was performed on a Hedera ODS-2 column using mobile phase of methanol - 10 mM ammonium acetate buffer solution containing 0.05% acetic acid (71:29, v/v) at a flow rate of 0.38 mL/min. Asperosaponin VI and the I.S. were eluted at 2.8 and 1.9 min, respectively, ionized in negative ion mode, and then detected by multiple reaction monitoring. The detection used the transitions of the deprotonated molecules at m/z 927.5 ⇢ 603.4 for asperosaponin VI and m/z 821.4 ⇢ 645.4 for glycyrrhizic acid (I.S.). The assay was linear over the concentration range of 0.15 – 700 ng/mL and was successfully applied to a pilot pharmacokinetic study in beagle dogs.

Use or Misuse of Ko143 for Bcrp Chemical Knockout Mice

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Chemical knockout approach represents a simple and useful tool to understand the role of Bcrp on blood-brain barrier by using Bcrp specific inhibitor. The most potent and selective BCRP inhibitor known so far is Ko143, the tetracyclic analogue of Fumitrmorgin C, which has been extensively used in *in vitro* cell assay. In spite of sporadic usage for *in vivo* experiment, the pharmacokinetic parameters for Ko143 remain largely unknown. Our data showed that Ko143 had moderate volume of distribution (1.85 L/kg), high clearance (85 mL/min/kg) and short half-life of 0.5 hr in mice after intravenous administration at 10mg/kg. Ko143 was rapidly hydrolyzed into its acid form in blood, thus acetonitrile must be used to stabilize Ko143 immediately after blood sample collection. The blood concentration was 420 nM at 1 hr post dose. In light that the plasma protein binding for Ko143 is 92-95%, the free drug concentration at 1 hr is similar to the IC_{90} (26nM). The brain penetration of Sorafenib, the specific BCRP substrates, increased at or less than 1.25 hr, but not at 2 and 4 hours after administration of Ko143 at 10mg/kg. In conclusion, brain penetration in Bcrp chemical knockout mice should be assessed within one hour post Ko143 administration.

Systemic and Cerebral Exposure to and Pharmacokinetics of Terpene Lactones and Flavonols after Dosing Standardized Extracts of *Ginkgo biloba* Leaves to Rats via Different Routes of Administration

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Ginkgo biloba leaves are used for treatment or prevention of cardiovascular disease, cerebral insufficiency, and peripheral arterial disease. The beneficial effects are believed to be attributed to ginkgo flavonol flavonoids (e.g., glycosides of quercetin, kaempferol, and isorhamnetin) and terpene lactones (e.g., bilobalide, ginkgolides A, B, C, and J). The aims of this study were to evaluate rat systemic and cerebral exposure to, pharmacokinetics, and disposition of these compounds after p.o., i.v. infusion, or i.m. administration of standardized preparations of *G. biloba* leaves, as well as to identify the key pathways affecting their exposure. After a single dose of GBE50 extract or ShuXueNing injection, the area under the plasma concentration-time curve (AUC) and peak plasma concentration of the ginkgo compounds were found to be increased directly at the selected dose levels. Oral bioavailability of terpene lactones after oral administration of the extract was significantly greater than that of the total flavonols converted from plasma samples using acidic hydrolysis pretreatment. The nanomolar-level concentrations of bilobalide, ginkgolides A, B, and C in rat hippocampus extracellular fluid were observed, but the flavonols were not measured. The absorbed intact flavonol glycosides were extensively excreted into bile by active mechanisms resulting in very lower systemic exposure, whereas the terpenoids were comparatively eliminated through urine and bile. Moreover, we found a significant correlation between the urinary recovery of the ginkgo compounds and its plasma AUC. This systematic study of exposure and disposition of ginkgo compounds render us the useful information for supporting the efficacy investigation.

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ADME

#4

Buspirone Metabolite Identification Using Comprehensive Survey Scan Modes and Intelligent Information Dependent Acquisition on A Hybrid Triple Quadrupole Linear Ion Trap Mass Spectrometer

Feng Qin, Jiehui Hu, Ping Du, and Yongming Xie
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Liquid chromatography coupled to mass spectrometry has been widely employed for metabolites identification in drug discovery and development field. Traditional workflow includes detection of metabolites using a full scan MS analysis, and identification of those metabolites using product ion scan (MS/MS). Due to the limited selectivity and sensitivity of full scan MS, as well as the lack of intelligent criteria to trigger product ion scan, many low abundant metabolites were missed in this workflow. Hybrid triple quadrupole linear ion trap mass spectrometer (QTRAP) has unique triple quadrupole scan modes that enable selective and sensitive metabolite detection, and ion trap scan modes that enable sensitive MS/MS acquisition, making it a good platform for drug metabolite identification. Here we report a comprehensive strategy using predictive-multiple reaction monitoring (p-MRM), neutral loss (NL) and precursor ion (Prec) as survey scans to detect buspirone metabolites after microsome incubation, and intelligent information dependent acquisition (IDA) to collect MS/MS data of the detected metabolites. Automatic method builder software named LightSight was used to generate all the methods and process the data. In total 92 metabolites were discovered, and many of which were structurally confirmed by the information rich MSMS spectra collected by the highly sensitive ion trap.

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POSTER ABSTRACT

ADME

#5

The Use of Multiple Fragmentation Methods for Small Molecule Characterization on an LTQ Orbitrap Velos

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Tandem mass spectrometry is a valuable tool for small molecule characterization due to its capability to yield a fragmentation fingerprint which reveals the structure information. The advanced MS/MS and MS^n capabilities of Collision Induced Dissociation (CID) and Higher-Energy Collision Dissociation (HCD) provide different ways to access fragmentation fingerprints. Such fragmentation capabilities can be combined with high resolution accurate mass to offer better data accuracy and confidence.

In this study, eleven small molecule standards were chosen with a variety of structure features, polarities, and molecular weights. These compounds were mixed and then spiked into biological matrices. An LTQ Orbitrap Velos mass spectrometer coupled to an Accela UHPLC system was used to compare the fragmentation efficiency of CID vs HCD. The sensitivity, spectral quality, and speed of the CID MS^n vs the HCD MS^2 for small molecule structural elucidation was compared and contrasted. Mass Frontier software was used for spectral annotation.

The results indicate that the sensitivity of CID and HCD MS/MS spectra are comparable. In general, 35% normalized collision energy for CID is efficient for fragmenting the majority of small molecule compounds, while the optimal collision energy for HCD varies depending on the structure features and molecular weight of the compounds. HCD is useful for the determination of low mass product ions and records ions resulting from multiple steps of collision, while the CID MSn preserves the structural linkage between fragments. About half of the compounds tested, under different energy level, a significant difference in fragmentation pattern was observed between CID vs HCD MS/MS spectra.

In conclusion, CID and HCD complement each other in providing diversified fragmentation pathways, generating information-rich, structurally diagnostic product ions. Combining both dissociation techniques together facilitates optimal, confident small molecule structure characterization, which is critical for metabolite identification, impurity and degradation product structure elucidation.

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POSTER ABSTRACT

ADME

#6

Analytical and Chemical Knowledge Management Software for Drug Metabolism Science

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Characterizing metabolite structures and metabolic pathways is essential to understand the potential biological implications of compounds in drug discovery. For over 15 years ACD/Labs has been facilitating discovery by offering cutting edge scientific software that is specialized in the management of chemical structures, reactions, analytical data as well as prediction of properties. Herein we describe a unique enterprise software platform that enables effective management and dissemination biotransformation pathways. This software accomodates discrete or indeterminate structures, associated analytical data and supporting information. The information is highly searchable and can be integrated with existing informatics infrastructure. It can also be extended to incorporate the many property prediction modules offered by ACD/Labs to better anticipate and avoid unwanted liabilities.

Platform for Predicting ADME/T and Activity

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In drug discovery, activity, ADME (Absorption, Distribution, Metabolism, Excretion) and Toxicity of compounds are very important criteria because they influence the drug levels, kinetics of drug exposure to the tissues and safety for people. It means that they influence the performance and pharmacological activity of the compound as a drug. It is well-known that, at the preliminary stage of drug discovery, in silico approach is one of the most economic ways to get the information about activity, ADME and Toxicity of compounds. Herein, a platform will be introduced, which can be used to predict pKa (logarithmic measure of the acid dissociation constant) related to absorption and distribution (CISOC-pKa); logP (measure of lipophilicity) related to the criteria mentioned (CISOC-logP); VD (volume of distribution) related to distribution; clearance (rate at which a substance is removed or cleared from the body by the kidneys and/or in renal dialysis) related to excretion; acute toxicity (CISOC-PSAT), carcinogenic toxicity (CISOC-PSCT), mutagenic toxicity (CISOC-PSMT), activity (PASS + herbs Information). All prediction systems in the platform have been employed in drug design, pesticide design and modernization of Traditional Chinese Medicine (TCM) successfully.

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Identification of Quaternary Ammonium-Linked Glucuronides of Morinidazole in Humans and Investigation of the Human UDP-Glucuronosyltransferases Involved

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Abstract Morinidazole (1-(2-methyl-5-nitro-1H-imidazol-1-yl)-3-morpholinopropan-2-ol), is an antimicrobial drug, currently in phase III clinical trials in China for the treatment of amoebas, trichomoniasis and anaerobic bacterial infections. The objectives of the present study were to identify the metabolites of morinidazole in healthy voluteers after iv administration and characterize the major enzymes involved. A total of 10 metabolites were detected in human plasma and urine by UPLC/Q-TOF mass spectrometry. The unchanged morinidazole, two glucuronides and a sulfate conjugate were predominant in plasma and urine. The glucuronides were resistant to hydrolysis by ß-glucuronidase from *Helix pomatia* at pH5 and pH7.4, but susceptible to hydrolysis by the enzyme from *Escherichia coli* at pH7.4. These conjuated metabolites were isolated from human urine by semi-preparative HPLC and characterized by NMR. Unexpectedly, the glucuronic acid moiety was linked through the nitrogen of morpholine ring of morinidazole, rather than the hydroxyl group, to form two diastereoisomeric quaternary ammonium-linked glucuronides. The plasma and urine concentrations of (R)-morinidazole glucuronide were markedly higher than those of (S)-entiomer. The two N^+-glucuronides were mainly excreted in human urine, accounting for 35% of the dose. The enzymes invloved in the glucuronidation were investigated by incubating morinidazole with human liver microsomes (HLMs) or recombinant UDP glucuronosyltransferase (UGT1A1, 1A4, 1A6, 1A9, 2B4, 2B7 and 2B15) fortified with UDP-glucuronic acid (UDPGA). The results showed that only UGT1A9, not UGT1A4, UGT1A3 or UGT2B10 which have been reported to catalyze N^+-glucuronidation of tertiary aliphatic amine, was responsible for the N^+-glucuronidation of morinidazole.

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POSTER ABSTRACT

ADME

#9

Establishing In Vitro Screen Capability for Time-dependent CYP Inhibition

Lisa Wang, Mingshi Guo, Jianfeng Huang, Yanjun Hu, Qiongqun Pan, Tingying Cao, Ping Li, Weiqing Chen*

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Time-dependent inhibition (TDI) was evaluated in vitro on five major cytochrome P450 (CYP) enzymes for furafylline, tienilic acid, paroxetine, mifepristone, and ticlopidine in both screen and kinetic characterization modes. The assays were conducted either in pooled human liver microsomes (HLM) or in recombinant human CYPs (rhCYP) after pre-incubation of the test compounds in the CYP enzymes in the presence or absence of NADPH (cofactor) followed by 10 ×dilution into the secondary incubation systems containing known substrates and the cofactor. CYP enzyme activities were determined based on metabolite production quantified by LC/MS/MS. A single time point (30-min) pre-incubation of furafylline, tienilic acid, paroxetine, mifepristone, and ticlopidine in HLM or rhCYP resulted in inhibition on CYP enzymes by 90% on CYP1A2, 80% on CYP2C9, 60% on CYP2D6, 54% on CYP3A4 and 54% on CYP2C19, respectively. The K_I and k_{inact} values were also determined for the test compounds in HLM or rhCYP after multiple pre-incubation time points at multiple concentrations under the current assay conditions. The results generated were comparable with those reported in the literature.

3D-QSAR Modeling for the Inhibitory Effects of Flavonoids on Recombinant Human CYP3A4 Activity

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Several beneficial properties have been attributed to flavonoids, including antioxidant, anti-inflammatory, and anticarcinogenic effects. The simultaneous administration of flavonoids and clinically used drugs may cause flavonoid–drug interactions. Inhibition of cytochrome P450 (CYP) is a major cause of drug-drug interactions. Interactions of flavonoids with CYP3A4, the predominant human hepatic and intestinal CYP, are of particular interest. To develop a three-dimensional quantitative structure-activity relationship (QSAR) model for predicting the flavonoid-drug interactions, the inhibitory effects of 18 flavonoids on CYP3A4 activity were investigated in recombinant human CYP3A4 (rhCYP3A4) incubation system. The data obtained were subjected to QSAR analysis by the linear modeling method genetic function algorithm (GFA) in Cerius^2 software. The proposed model was able to predict drug–CYP3A4 interactions with reasonable accuracy, and it indicated that the inhibitory activity was principally governed by spatial descriptors (Density, Area, and PMI-mag), electronic descriptor (Dipole-mag), and thermodynamic descriptor (AlogP98). Density and area are important positive factors of the inhibitory activities, while PMI-mag shows weak negative correlation. Dipole properties has been identified as having utility, possibly relating to the open active site of CYP3A4. The QSAR+ descriptor ALogP98 also emerged as an important descriptor, which suggest that the flavonoid analogues may bind significantly to the hydrophobic pockets of the CYP3A4 active site, and the octanol/water partition coefficient of molecular may be important factors. These results will guide to design the synthesis of new flavonoid analogues with less CYP3A4-inhibitory properties and open a way for in silico prediction of xenobiotic inhibition of CYP3A4.

Key words: flavonoid; rhCYP3A4; 3D-QSAR; flavonoid–drug interactions

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POSTER ABSTRACT

ADME

#11

Validation of a Single Derivative LC-MS/MS Method for the Determination of Glucose in Human Plasma with [$^{13}C_6$]D-Glucose as a Calibrator

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For development of drugs to treat diseases like diabetes, it is important to monitor the levels of endogenous glucose or other monosaccharides in human plasma. Here we report a novel surrogate approach capable of quantifying D-glucose from human plasma based on derivatization LC-MS/MS method. [$^{13}C_6$]D-glucose was used for standard and QC preparation and [$^{13}C_6$,D_7]D-glucose was used as the internal standard (IS). Plasma samples were extracted by protein precipitation; the extracts were evaporated to dryness, reconstituted with water, and followed by incubation with derivatization reagent at 37ºC for 2 h prior to dilution for LC-MS/MS analysis. The yield of the derivatization and method recovery were evaluated using [^{14}C]D-glucose. The precursor and product ions were monitored at m/z 358/284 for derivatized D-glucose, 364/290 for derivatized [$^{13}C_6$]D-glucose, and 371/297 for derivatized [$^{13}C_6$-D_7]D-glucose. This approach employs a unique derivatization procedure by which only glucose gives a good derivative product, while the recovery of most isomers very low. In addition, the mass sensitivity was increased dramatically, and the matrix effect is minimized. The mass response ratio of D-glucose and [$^{13}C_6$]D-glucose was determined to generate a conversion factor for the calculation of D-glucose concentration based on the calibration curve of [$^{13}C_6$]D-glucose. The method has been successfully validated with an excellent linearity obtained in a range from 0.100 mg/mL to 5.00 mg/mL of [$^{13}C_6$] D-glucose in plasma ($R^2 \geq 0.999$). the inter-day precision (CV%) and accuracy (RE%) for all QC plasma samples, including LLOQ, were ≤3.08% and ≤2.50%, respectively. Plasma samples were stable after three freeze/thaw cycles and ambient temperature storage for up to 24 h.

Evidence for Bioactivation of 5-Caffeoylquinic Acid to ortho-Benzoquinone In Vitro

Cen Xie, Dafang Zhong, Xiaoyan Chen*

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5-Caffeoylquinic acid (5-CQA) is a major active ingredient in some Chinese herbal injections used to treat common colds and upper respiratory tract infections. Occasional anaphylaxis has been reported in their clinical use. The major objective of the present study was to identify reactive metabolites of 5-CQA. When incubated 5-CQA with GSH-supplemented HLMs, two types of GSH adducts were characterized by UPLC/Q-TOF MS and confirmed by chemical synthesis and NMR. They were derived from the 1,4-addition of GSH at C-2 position of *ortho*-benzoquinone intermediate (M1), or directly at C-7 position of ∂,ß-unsaturated carbonyl group of parent (M2). The two-electron transfer oxidation of catechol to *ortho*-benzoquinone was partially P450-mediated with 3A4 and 2E1 as two principal catalyzing enzymes. In SAM- and GSH-supplemented human S9 incubations, the major metabolites were *O*-methyl-5-CQAs (M3), with only small amount of M1 detected. However, the formation of M1 was reversibly fortified after inhibiting COMT activity by tolcapone, a COMT inhibitor. This finding indicates that *O*-methylation of 5-CQA *in vivo* may downgrade its oxidative capability to form *ortho*-benzoquinone. Furthermore, the bioactivation of 5- CQA was investigated in the incubations of HLMs spiked with cumene hydroperoxide, or myeloperoxidase (MPO) from human leukocytes, using GSH as trapping agent. As a result, the production of M1 was markedly enhanced, and the formation of bi- and tri-GSH adducts (M5, M6, and M7) was also observed. These results suggest that under the oxidizing or inflammatory conditions, 5-CQA may be more susceptible to facile bioactivation to reactive *ortho*-benzoquinone species following two sequential single-electron transfer oxidations.

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POSTER ABSTRACT

ADME

#13

The Development of A Cell-based Model for Drug Passive Transport Screening

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Purpose: RRCK is a monoclonal isolate of MDCK obtained using FACS sorting for P-gp using the P-gp substrate Cacein-AM. The mRNA level for canine P-gp was approximately 200-fold lower than that seen in MDCK. Due to its low transporter activity, RRCK was used to study drug passive transport. Here, the endogenous P-gp activity in RRCK was further investigated using 9 marketed drugs. By cassette dosing of test drugs, P-gp-mediated drug-drug interactions were also investigated.

Methods: RRCK as well as MDCK, MDCK-MDR1, and Caco-2 were plated to Transwell plates, and cultured using standard techniques to obtain polarized epithelial monolayers. The bi-directional transport was conducted in triplicate, and the samples were collected at time 0 and the end of incubation. Test compound mass was determined using LC/MS/MS analysis based on the peak area ratio of test compound to internal standard. The monolayer integrity was measured using Lucifer yellow after transport experiments.

Results: The results showed that low (atenolol and fenoterol) and high (metoprolol, propranolol and triprolipidine) permeable compounds showed differentiable and consistent Papp values and efflux ratio (ER) of <2.0 among Caco-2, MDCK-MDR1, MDCK and RRCK. The substrates (CsA, digoxin, quinidine and verapamil) of efflux transporters were only well-correlated in Caco-2 and MDCK-MDR1 cells, as expected due to the functionally active P-gp in these cell systems. The ER values of CsA, digoxin and quinidine were 4-8 in MDCK but < 2.0 in RRCK. Verapamil showed negligible ER values both in MDCK and RRCK. The sharp decrease of ER values in RRCK indicated that RRCK had almost null functional activity of endogenous P- gp.

By cassette dosing, the permeation of atenolol, propranolol, fenoterol and metoprolol was not influenced in RRCK bi- directional transport; even dosed together with efflux substrates and inhibitors, the Papp and ER values of these passive transport makers were still consistent to those at single dosing. At cassette dosing, the permeation and ER values of CsA and digoxin were not influenced by passive transport markers and the substrates and inhibitors of efflux transporters. Compared to those at single dosing, the permeation of quinidine and verapamil had 2~3 fold increase, but ER values were still close to 1.0. The increase might be caused by the variations of instrumental detections, bio-incubation, etc.

The data of ~1000 Pfizer and marketed compounds in practice showed that only 6% of ~ 1000 Pfizer and marketed compounds showed efflux ratio (ER) of >1.7 in RRCK while 41% and 69% in MDCK and MDCK-MDR1, respectively. In addition, the maximum ER value observed in RRCK was 3.4 while it was 18.4 and 117 in MDCK and MDCK-MDR1, respectively. These results further confirm the negligible functional activity of P-gp in RRCK

Conclusion: In summary, the low to non-existent efflux transport of marketed and Pfizer compounds showed that RRCK has a negligible P-gp functional activity. Additionally, passive permeability results from Caco-2, MDCK and RRCK aligned well. Taken together we conclude that RRCK is a suitable model to study drug passive transport.

Rational Use of Rat In Vitro Blood-Brain Barrier Co-culture Model for Central Nervous System Drug Discovery

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Primary co-culture of brain microvessel endothelial cells (BMECs) and astrocytes represents the most physiologically relevant *in vitro* blood–brain barrier (BBB) model that retains many of the unique BBB biochemical properties. A rat primary co-culture BBB model with high transendothelial electrical resistance and low permeability of lucifer yellow (< 40 nm/s) has been set up. Temporal mRNA expression of major transporters and receptors in rBMEC on porous inserts was examined by qRT-PCR and compared with freshly isolated rat brain microvessels. The mRNA expression of *bcrp-1, mrp1* and insulin receptor was best retained at day-6 (mRNA level >80% of freshly isolated brain microvessel) whereas the expression of uptake transporters *(oatp1a4, oat1, oat3* and *Glut-1*), transferrin receptor 1 and Lrp-1 was rapidly lost in the first 2 days and not exceeded 5% of freshly isolated brain microvessel at day-6. *Mdr1b* was the only gene found to be upregulated. The permeability of 5 CNS marketed drugs was consistent across three independent experiments (RSD < 21%). Pgp and Bcrp-1 functions were evidenced by vectorial transport of amprenavir and dantrolene with efflux ratio of 6.1 and 7.7, respectively. Strong correlations (R^2 = 0.8) were observed between permeability obtained from the *in vitro* BBB model and rat *in situ* brain perfusion for 12 central nervous system (CNS) marketed drugs. In summary, despite of altered expression of BBB transporters and receptors, BMEC remains a robust and useful in *vitro* BBB model for CNS drug discovery.

Analysis of the Metabolites of Buspirone in Hepatocytes Using a Triple Quadrupole Mass Spectrometer and Ion Trap Technology

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The liver is the most important site of drug metabolism in the body. Approximately 60% of marketed compounds are cleared by hepatic CYP-mediated metabolism. Cryopreserved hepatocytes provide a useful *in vitro* model for drug metabolism. However, humans differ from animals with regards to isoform composition, expression and catalytic activities of drug metabolizing enzymes. Therefore, it's preferable to conduct hepatocytes stability studies across multiple species to predict the metabolite profile in human. In the present study, Buspirone was incubated with human, dog, rat and mouse hepatocytes and was found to undergo N-dealkylation, oxidation and glucuronidation. Totally 11 metabolites were identified by using hybrid quadrupole/linear ion trap, which combines the capabilities of a triple quadrupole mass spectrometer and ion trap technology on a single platform. Among them, the despyrimidine metabolite was only present in rat hepatocytes, whereas bis-oxidation on azaspirodecane of Buspirone existed in human hepatocytes. While these four species have much in common in terms of metabolite structures, there existed appropriate interspecies differences.

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POSTER ABSTRACT

ADME

#16

MassHunter Study Manager: An Intelligent Software Tool to Automate LC/MS Workflow for DMPK Studies

Yuqin Dai, Doug McIntyre, and Keith Waddell
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High performance Liquid Chromatography coupled to triple quadrupole mass spectrometry has proven to be a powerful tool in providing rapid quantitation of drugs and their metabolites for DMPK studies. However, in the early stage of drug discovery, thousands of new chemical entities (NCE) may be screened for ADME assessment; the MRM optimization of a large set of compounds and data processing often become a bottleneck in the process, so further streamline of LC/MS workflow is crucial. In this work, an intelligent software tool named MassHunter Study Manager is presented. This software automates all steps of LC/MS workflow from compound MRM optimization, method creation, and data acquisition throughout quantitation. The result is a significant reduction in labor-intensive manual steps and an increase in the efficient use of resources. An example of using MassHunter Study Manager for in vitro metabolic stability studies is successfully illustrated for a large set of compounds. This example will demonstrate the automation and time saving potential of the software.

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POSTER ABSTRACT

ADME

#17

The End of MRM: Ultrafast SPE-TOF Analysis Streamlines Workflow and Increases Throughput of ADME Assays

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The throughput of in vitro ADME analysis is gaining an almost equal footing with data quality as a determinant of laboratory workflow. These assays have traditionally utilized tandem MS, which is limited by its requisite MRM method development that requires several minutes per sample of processing time. The use of accurate mass offered by high resolution (time of flight: TOF) mass spectrometers, which eliminates the need for MRM optimization, in conjunction with a high throughput solid phase extraction (SPE) system was investigated to assess its ability to enable a faster and more efficient assay analysis workflow. We compared the assay results for a panel of in vitro ADME assays (CYP inhibition, metabolic stability, PAMPA and plasma protein binding) using ultrafast SPE-MS/MS and SPE-TOF systems for analysis. A chemically diverse set of 50 compounds was used for each assay and all analyses utilized the same generic SPE conditions. All SPE-TOF analyses were performed using the same generic MS conditions with a RapidFire 360 interfaced to an Agilent 6530 Q-TOF run in ESI-TOF mode. The experimental results obtained for all four of the ADME assays by each analysis system were comparable (R2 >0.9). Using a generic SPE condition, the ultrafast SPE-MS systems consistently produced sample analysis cycle times of 7 seconds/sample with no changes to standard laboratory workflow and no significant sample carryover. Using a generic MS method, the TOF analysis gave comparable results to conventional triple quadruple analysis. Assay results with the SPE-TOF system were comparable to the SPE-MS/MS system for a variety of ADME assays indicating that MRM method development could be eliminated for these assays providing a significant increase in efficiency of laboratory workflow. The SPE-TOF system enabled faster and more efficient assay data analysis across a panel of ADME assays without compromising the quality of results.

Determination of CF in Rat Plasma by LC–MS/MS: Application to Pharmacokinetic Study

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CF was a diterpenoids compound isolated from the *Coleus forskohlii Briq*. Its anticancer activity has been performed by using many experimental models, including in vitro test systems and whole animals. A sensitive, selective and reproducible LC-MS/MS method for determination of CF in rat plasma was developed and validated. After extraction with Methyl tert-Butyl ether, Chromatographic separation was performed on a BetaBasic-C18 column with the mobile phase consisting of water 0.1% formic acid-acetonitrile in the gradient elution mode at a flow-rate of 0.2ml/min. A tandem mass spectrometer equipped with electrospray ionization source was used as detector and operated in the positive ion mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions m/z 411m/z ⇢ 375 and m/z 285 ⇢ m/z 193 for CF and diazepam(IS) respectively. The linear calibration curve was obtained in the concentration range of 0.5-1000ng/ml. The intra-and inter-day precision was measured to be below 14%. The relative error was between -0.1% and 2.5%. This method was successfully applied to the pharmacokinetic study after an intravenous administration of CF in rats. The ration of the area under concentration-time curve (AUC) in rat is 18.38, 137.44,1626.58 ng/ml at three dose of 0.2,1,5mg/kg for CF after intravenous administration ,respectively. The mean t1/2 was 0.32, 0.27 and 0.69h.These results exhibited a nonlinear relationship between AUC and concentration.

Plasma Protein Binding of the Investigational Anticancer Agent S-propargyl-cysteine

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S-propargyl-cysteine (SPRC) is a novel sulfur-containing amino acid derivative currently being tested in pre-clinical stage as an anticancer candidate. Here, studies were performed to evaluate plasma protein binding to SPRC and evaluate species differences in free drug levels. The drug concentrations were determined by using a validated LC/MS/MS method. The ultrafiltration method used to characterize plasma protein binding to SPRC led to negative results in both human (-35.2% ± 3.16%) and dog plasma (-39.5% ± 15.3%), while left positive results in rat plasma (11.4% ± 5.25%). After ruling out the possibilities of non-specific binding of the device and low solubility of the compound, we tested it again by using equilibrium dialysis method. Very low protein binding was observed for SPRC in human (-5.62% ± 7.66%), dog (-1.92% ± 2.96%) and rat (7.02% ± 3.61%) plasma. Since the protein binding to the positive control compound, warfarin, was within the normal range (98.4% ± 1.10%), which indicated the reliability of the study, it could be concluded that very little SPRC is bound to plasma proteins in those three species. Furthermore, since the method of ultrafiltration might have contributed to the extreme data in the case of SPRC, it might not be proper to test the plasma protein binding of low-bound compounds.

Inter-species Comparison of Metabolite Profiles for Propranalol in Rat and Human Liver Microsomes

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Propranolol, a ß-adrenergic receptor antagonist is widely used for the treatment of hypertension, ischemic heart disease and certain arrhythmias. The biotransformation of propranalol has been studied previously in different in vitro and/or in vivo systems. However, certain ambiguity on the inter-species differences on its metabolism remains. The in vitro metabolism of propranalol in rat and human liver microsomes has been compared in current study. Propranalol was observed as the substrate for P450 catalyzed hydroxylation and dealkylation in both rat and human liver microsomes. Among the identified mono-oxidated metabolites, two mono-oxidated metabolites were only detected in human liver microsomes, while the other two were only detected in rat liver microsomes. All these four mono-oxidated metabolites were structurally related isomers but eluted at different retention times, which demonstrated that the locations of the mono-oxidation occurred on propranolol in rat liver microsomes were different from those in human liver microsomes. To the best of our knowledge, this is the first research conducted to investigate the inter-species comparison of CYP450 mediated mono-oxidated metabolites on propranolol formed between rat and human liver microsomes

POSTER ABSTRACT

ADME

#21

Understanding the Role of Quality by Design in Chromatographic Method Development

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Quality by Design (QbD) has become popular within the pharmaceutical industry and its application to process analytical technology (PAT) and drug manufacturing is rapidly increasing. At the heart of QbD lies several principles, all leading toward the goal of building in quality right from the earliest stages of drug discovery, including knowledge retention, elimination of errors, and increased experimental scope. To be successful, QbD must be applied at every stage in the development and manufacturing process; however, one can consider processes within drug discovery on an individual basis to apply QbD principles. One such process is the development of chromatographic methods for impurities and degradant studies. Ensuring both robustness and optimization from an efficiency standpoint is time-consuming and difficult. Generally method development is carried out using a trial-and-error approach, and requires a large amount of manual data interpretation. However, QbD principles can be directly applied to this process, resulting in better, more robust separations. Furthermore, quality is achieved when QbD is applied to the stability study process by producing well developed, traceable, error free results, and finally into the drug product by bringing novel products to market faster, and more cost effectively.

Simultaneous Determination of Lidamycin Enediyne Chromophore (LDC) and its Aromatized Derivative (LDCA) Using Puerarin as Internal Standard in Plasma by LC-MS/MS

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Lidamycin (LDM), a promising enediyne antitumor antibiotic, was quantified by detecting lidamycin enediyne chromophore (LDC) using liquid chromatography–tandem mass spectrometry (LC-MS/MS) for the first time. A simple, rapid, and reliable method was developed and validated to determine LDC and its aromatized derivative (LDCA) simultaneously in plasma. Puerarin was used as internal standard (IS), and biological samples were pretreated with one-step precipitation by acetonitrile, Separation was achieved on a reverse phase C8 column with a mobile phase composed of methanol and water containing 5mM ammonium acetate at pH 3.5 in gradient elution mode. Detection was performed on a triple quadrupole tandem mass spectrometer using electrospray ionization (ESI) by multiple reaction monitoring (MRM) in the negative ion mode. Good linearity was obtained over the concentration range of 0.2~100 μg/ml for LDM. Reliable precision and accuracy were validated by R.S.D. % values in range of 2.6~13.0% and R.E. % values between -4.6% and 3.8%, respectively. In addition, no specificity and matrix effect were observed. The recovery was found to be 99.2~111.0%, and stability in various conditions were found acceptable. This method was applied in preclinical pharmacokinetic studies for routine monitoring of LDM level in plasma.

Keywords: LC–MS/MS; lidamycin; enediyne chromophore; aromatizatized derivative; Pharmacokinetics;

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Metabolism and Disposition of [^{14}C]Dapagliflozin in Humans: Identification of Major Metabolites and Enzyme Responsible for Major Clearance Pathway

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Dapagliflozin a rationally designed, potent and selective inhibitor of the renal sodium-glucose co-transporter, SGLT2, is being developed as an orally active agent for the treatment of type 2 diabetes mellitus. The objectives of the current studies were to determine metabolism and disposition of Dapagliflozin in humans as well as enzyme responsible for the major clearance pathway. [^{14}C] Dapagliflozin was given orally to humans (50 mg, 6 subjects). Plasma, urine and feces were collected for mass balance analysis and metabolite profiling and identification. The recovery of radioactivity in urine and feces was 74.9 % and 21.0%, respectively. LC/radiodetection/MS analyses allowed identification of multiple metabolites. The prominent circulating components in plasma were dapagliflozin and dapagliflozin 3-O-glucuronide (M15). M15 was also the single dominant radioactive component in human urine. The structure of M15 was determined by MS and 2D-NMR. The formation of M15 followed by renal execretion, which accounted for 60.6% of the dose, was the major drug clearance pathway in humans. A reaction phenotyping study of M15 formation was conducted with expressed human UGT, human liver microsomes in the presence of individual chemical inhibitors of UGTs and a panel of human liver microsomes (N = 15) with various levels of UGT1A9 activity. Results indicated that UGT1A9 was the sole tested UGT enzyme that exhibited significant catalytic activity for the formation of M15.

Key word: Dapagliflozin, SGLT2, Metabolism, Metabolic profile, Mass Balance, Human, Glucuronide Conjugates, Animal species

Metabolite Identification of Clozapine in Rat and Human Liver Microsomes

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Clozapine, an atypical neuroleptic drug, is used for the treatment of resistant schizophrenic patients who do not respond to typical neuroleptic therapy. The biotransformation of clozapine has been studied previously in different in vitro and/or in vivo systems. However, inconsistent results on the major metabolites of this compound by CYP450 have been reported. In addition, certain ambiguity remains for several other metabolites. In present study, the in vitro metabolism of clozapine in rat and human liver microsomes has been investigated and compared. The major metabolites of clozapine in both rat and human liver microsomes were identified as demethyl clozapine and clozapine N-oxide. Clozapine was also extensively transformed into a wide array of metabolites including multi-site mono-oxidation, demethylation with mono-oxidation, reduction of piperazine ring with mono-oxidation, and reduction of piperazine ring with di-oxidation in rat and human liver microsomes. Further in vivo studies need to be tested in order to correlate the results between in vitro and in vivo data.

Pharmacokinetics, Mass Balance, Tissue Distribution and Metabolism of [^{14}C]-Acetaminophen in Male Rats

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Acetaminophen is a widely used, nonprescription analgesic and antipyretic drug; however in over dose, it can cause severe hepatic and renal celular necrosis. This study investigated its metabolic fate in rats using radiolabeled acetaminophen. Following a 20 mg/kg (20 μCi/kg) single oral administration of [^{14}C] acetaminophen to male Sprague-Dawley rats, urine, feces and cage wash were collected up to 168 h post-dose. A total of 98.44% of radioactivity was recovered, with urine containing 79.93% of the radioactive dose and feces containing 6.76%. Plasma samples were collected for pharmacokinetics study. The peak plasma concentration (C_{max}= 28.5 μg eq/g) was achieved at 0.25 h, with a short elimination half life of 2.59 h, and plasma protein binding of acetaminophen was negligible. The [^{14}C]acetaminophen radioactivity was widely distributed in most tissues with high concentrations observed in gastrointestinal (GI) tract, kidneys, liver, and thyroid, suggesting that acetaminophen was absorbed rapidly from the GI tract, metabolized in the liver and excreted by the kidney. Acetaminophen was metabolized extensively. The primary route of elimination was excretion of sulfate conjugates into urine (86.0%), followed by renal excretion of glucuronide conjugates (6.26%) and unchanged drug (<5%), only a small amount (1.19%) was metabolized into the reactive intermediate *N*-acetyl-*p*-benzo-quinone imine (NAPQI), which was then conjugated with glutathione and finally excreted as mercapturic acid. The metabolites in feces were minor except a nonpolar metabolite M4, which was first confirmed by a radiolabeled study and identified as a dimer of acetaminophen and accounted for 77.86% of all feces exposure.

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ADME

#26

Purification of Drug by FLASH Chromatography with Amide Bonded Silica Media

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In Strong polarity drug purification , the two problems were that the drug is usually convert to salt for alkality is unstabled,and the drug was in ionic form. There was no retention in reversed phase chromatography, such as C18, and short life of column efficiency would be found in normal phase chromatography suce as silica or NH2. In order to solve the problems to save time and cost, a novel media material – Amide Bonded Silica - was made by Bonna-Agela Technologies, Amide series media was worked in the hydrophilic interaction chromatography mode, significantly efficiency was gain when purificating the strong polarity drugs-especially strong water-soluble drugs. An application of slimming drug showed the advantages of the Amide series media when compared with the traditional silice media under the same detection conditions: (1) Sample loading was up to 35 mg/g (sample/ media weight ratio) while 15 mg/g of Silica media; (2)Recovery up to 48% while 23% of Silica media;(3) Column life up to 50 times while 5 times of Silica media. We can draw some conclusions from that:Amide series media can be got good performance,such as sample loading,recovery, column life, when purificate the strong polarity and water soluble compounds.

POSTER ABSTRACT

ADME

#27

The Comparison of Methods of Protein Precipitation, SLE and MAS to Treat Dexamethasone in Plasma

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Phospholipids is one of the crucial endogenous interfering substances in plasma, and often cause matrix effect, affecting the accuracy of results. The latest plasma samples treatment products, SLE and MAS, can remove more than 99% of phospholipids in plasma. The packing material of SLE (Solid Supported Liquid/Liquid Extraction) is diatomaceous earth, which has a large inertia adsorption surface, which can absorb phospholipids in plasma. The packing material of MAS (Multi-Adsorption Reverse SPE) is tertiary ammonium resin or benzene sulfonic acid resin, either of which can absorb phospholipids by ion pairing, RP, NP, hydrogen bonding, etc. This paper separately used the methods of traditional protein precipitation, protein precipitation plate, SLE and MAS to treat dexamethasone in plasma. The results showed that the responses of SLE and MAS are about five times of protein precipitation. The reason might be that the method of protein precipitation can't effectively remove phospholipids, causing serious matrix interference. While the samples treated by SLE and MAS contain little phospholipids, avoiding matrix effect effectively. Meanwhile, protein precipitation plate, SLE plate and MAS plate are all 96 well plate, and they can treat samples with high throughput, saving the time of the sample treatment.

POSTER ABSTRACT

ADME

#28

Studies on Chemical Components of Effective Fraction from Angelica Dahurica

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A method to develop the chemical components from effective fraction, chemical components has been studied. Take the proper standard sample of Imperatorin-dissolved in methanol with the concentration of 0.5mg/mL. Take the proper quantity of alcohol extraction, water soluble extraction and ethyl acetate extraction from Angelica dahurica, reconstitute in methanol, each of the sample concentration is 2mg/mL respectively, filtered with 0.45μm syringe filter. Inject 10μL of standard and sample solvent respectively into the HPLC instrument and analyze. Comparison the chromatograph of different solvent extraction, we can easily find that Venusil XBP C18 (L)can separate all the coumarin completely in one run, and different parts of extraction maybe show various biological activity which should be further studied.

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Pharmaceutical Sciences

#29

Direct Analysis in Real Time-Mass Spectrometry (DART-MS) for Salt Characterization in Solid Form

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At the first time, Direct Analysis in Real Time (DART)-MS has been applied to solve the difficult problem for salt characterization in the original form by providing the information of Mw of salt. Over 10 organic salts have been investigated by DART-MS and the result shows that DART-MS for salt Mw is applicable for broad kind of salts to get confirmative information about salt formation and stoichiometry. To choose optimal salt form of API is a key factor contributed to success of the drug. However, the characterization of salt such as salt formation and stoichiometry is very difficult, especially for amorphous salt, if without destroying the solid form. Currently, pharmacist must use multiple indicative methods such as XRPD, DSC, TGA, Elemental analysis, ssNMR and Ion chromatography to know the possibility of salt formation and stoichiometry. Therefore, we proposed to use DART-MS to solve this difficult problem by showing Mw of salt to provide the direct information about salt formation and stoichiometry. More importantly, this method compared to other methods has the following unique advantages: 1) test sample in original solid form, 2) only micron gram of salt is needed which is more suitable for screening stage, 3) The test procedure is very fast without sample preparation.This new application may have great impact on pharmaceutical industry by providing the new powerful tool for direct salt characterization prior to other existing indicative characterization methods. Furthermore, from this new application, DART-MS/MS represents a remarkable opportunity to understanding the ionization mechanism of the compounds.

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ADME

#30

Accurate Determination of the Anticancer Prodrug Simmitecan and its Active Metabolite Chimmitecan in Various Plasma Samples Based on Immediate Deactivation of Blood Carboxylesterases

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Simmitecan (L-P) is an anticancer ester prodrug, which involves activation to chimmitecan (L-2-Z). In the current study, a liquid chromatography/tandem mass spectrometry-based method was developed for simultaneous determination of L-P and L-2-Z in various plasma samples. Because L-P is rapidly converted to L-2-Z by blood carboxylesterase during and after sampling, which hampers the accurate determination of L-P and L-2-Z in the biological samples, different carboxylesterase inhibitors were tested. As a result, bis(4-nitrophenyl)phosphate gave the best results with respect to inhibitory capability, hemolysis, and matrix effects and was used to deactivate blood carboxylesterases when sampling. The plasma samples were precipitated with acetonitrile and the resulting supernatants were separated using a pulse gradient method on a C18 column. Irinotecan and camptothecin were used as internal standards for quantification of L-P and L-2-Z, respectively. Protonated L-P, L-2-Z and their internal standards were generated by electrospray ionization and their precursor-product ion pairs (m/z 599 ⇢ 124, 405 ⇢ 361, 587 ⇢ 195, and 349 ⇢ 305, respectively) were used for measurement. The newly developed bioanalytical assay processed favorable accuracy and precision with lower limits of quantification of 2.1 nM for L-P and 3.4 nM for L-2-Z, and was successfully applied to pharmacokinetic studies in tumor-bearing nude mice, rats, and dogs. There are substantial species differences in the ester activity. The experimental strategies illustrated in our report may be adopted for measurement of other prodrugs (including irinotecan) or drugs subject to ester hydrolysis, as well as their metabolites, in biological matrices.

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ADME

#31

Biodistribution of Ad5-001 following Single Intramuscular Injection in Mice with Quantification Polymerase Chain Reaction

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Objective: To assess the distribution following single administration of Ad5-001.

Method: Ad5-001 was single intramuscular administered at the dosage of 10^{11} v. p./0.1ml/animal into C57BL/6J mice. Ten animals were euthanized and different tissues were collected at different time point till 84 days after dosing. The Ad5-001 genomic DNA was assessed with Q-PCR.

Results: One day after administration, Ad5-001 genomic DNA was detected mainly in the injected muscle (142987 ±113522 copies/μg DNA), groin lymph node on the injected side (3273 ± 3735 copies/μg DNA), liver (239 ± 93 copies/μg DNA), spleen (89 ± 38 copies/μg DNA) and lung (15 ± 17 copies/μg DNA). Only a few of Ad5-001 genomic DNA distributed in blood (1/5 of female and 5/5 of male) and muscle at opposite site of injection (1/5 of female and 4/5 of male). And no Ad5-001 genomic DNA was detected in heart, kidney, brain, testes/ovaries. Then the amount of Ad5-001 genomic DNA in injected muscle gradually declined. However, the level of Ad5-001 genomic DNA increased till 5 days after administration and then decreased. On 84 days after administration, Ad5-001 genomic DNA could still be detected in injected muscle (2888 ± 1269 copies/μg DNA), groin lymph node (236 ± 84 copies/μg DNA), liver (26 ± 9 copies/μg DNA) and spleen (18 ± 5 copies/μg DNA).

Conclusions: Following single intramuscular administration of 10^{11} v. p./0.1ml/animal of Ad5-001, genomic DNA mainly distributed in the injected muscle and could still be detected in injected muscle, groin lymph node, liver and spleen on 84 days after administration.

Keywords: Biodistribution, Adenovirus, Quantification Polymerase Chain Reaction, Gene therapy

Intestinal Absorption and Presystemic Elimination of Various Chemical Constituents Present in GBE50 Extract, a Standardized Extract of *Ginkgo Biloba* Leaves

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The nature and level of systemic exposure to active herbal constituents will profoundly affect their bioactivities at action sites, which is fundamental in understanding their roles in the overall beneficial effects of an herbal medicine. The objective of this study is to gain a full picture of the systemic exposure to various putatively active ginkgo constituents after p.o. administration of GBE50 extract, a standardized extract of *Ginkgo biloba* leaves, to rats and understanding of the relevant mechanisms governing the intestinal absorption and presystemic elimination. To define the ginkgo compounds to be studied, literature informatics-guided chemical profiling revealed that GBE50 extract contained 71 ginkgo constituents, including terpene lactones, flavonols, flavones, an isoflavone, biflavones, flavanols, and carboxylic acids, at levels ranging from 0.01 to 55.3 mg/g. Among the ginkgo constituents were the terpene lactones and the flavonol glycosides that were significantly measurable in the postadministrative rat plasma. The intestinal absorption of terpene lactones appeared to be dictated by their intermediate membrane permeability, while the influences of MDR-1- and MRP-2-mediated intestinal efflux and the presystemic metabolism and biliary excretion might be limited. Because of their deglycosylation absent in the small intestine and relatively slow presystemic elimination, many intact flavonol glycosides could be absorbed albeit to a limited extent. Colonic deglycosylation of the flavonol glycosides occurred and the glucuronides of flavonol aglycones were also measured in the rat plasma. Although some biflavones had relatively high abundance in GBE50 extract, these ginkgo constituents were not measured in the rat plasma because of their poor solubility and poor permeability that hindered the intestinal absorption. The levels of the remaining ginkgo constituents in GBE50 extract were too low to be measured in the rat plasma. The current study enabled us to better understand the nature of systemic exposure to ginkgo compounds after p.o. administration of GBE50 extract and to more precisely implement multicomponent PK study of the extract.

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ADME

#33

The Interplay of Endogenous Drug Transporters in Caco-2, MDCK-MDR1 and MDCK

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Purpose: Numerous drug transporters are endogenously expressed in Caco-2 and MDCK cells, such as MDR1, BCRP, MRP2, etc, and function in overlapping way to determine drug transport across cell monolayers. Here, 35 marketed drugs plus the inhibition methods were used to study the interplay of endogenous drug transporters in Caco-2, MDCK-MDR1 and MDCK cells.

Methods: Caco-2 MDCK-MDR1 and MDCK cells were plated into Transwell-96 well plates, and cultured to form differentiated epithelial monolayers. Bi-directional transports were conducted to assess the permeability, efflux ratio (ER) and the correlation among three assays. Some drugs were randomly picked out to further examine the interactions with drug transporters using inhibition experiments in Caco-2 and MDCK-MDR1. Lucifer yellow was used to confirm the monolayer integrity after transport experiments.

Results: The results showed that the drugs were well differentiated into low, moderate and high permeability and ER values. The permeation (Papp values) showed poor correlation between Caco-2 and MDCK-MDR1 (R2=0.418), Caco-2 and MDCK (R2=0.080) and MDCK-MDR1 and MDCK (R2=0.495).

To further extrapolate the potential mechanisms, some drugs were picked out to conduct inhibitor studies. When exposed to CsA at 50 μM, taurocholate showed sharp Papp increase both in Caco-2 and MDCK-MDR1 while salicylamide not. The inhibition possibly released the limitations of implicated transporters for taurocholate transport such as ASBT, MDR1, OATP1A2, although the inhibiting effects vary greatly between Caco-2 and MDCK-MDR1. Methotrexate and sulfasalazine showed high ER values in Caco-2 but negligible in MDCK- MDR1, indicating the weak interaction with MDR1. At the exposure to transporter inhibitors, elacridar for BCRP & MDR1 and imatinib for BCRP, the alterations of the permeation exposed to showed that methotrexate interacted mainly with BCRP while sulfasalazine with BCRP and others instead of MDR1. Prednisone, triiodothyronine and topotecan showed higher ER values in Caco-2 while slightly lower in MDCK-MDR1, indicating the potential roles of more besides MDR1. The inhibition studies indicated that MDR1, BCRP and even more transporters interplay together to cause the asymmetric transport.

Conclusion: In summary, drug transport correlated poorly due to the overlapping expression and functioning of endogenous drug transporters among Caco-2, MDCK-MDR1. More than MDR1 and BCRP are implicated with drug transport in Caco-2 and MDCK cells.

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ADME

#34

Evaluation of ionic Liquid Stationary Phases for GC-MS and GC×GC Analyses of Fatty Acids in Marine Biota

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Ionic liquid stationary phases were tested for one dimensional gas chromatography-mass spectrometry (GC-MS) and comprehensive two dimensional gas chromatography (GC×GC) of fatty acid methyl esters from algae. In comparison with polyethylene glycol and cyanopropyl substituted polar stationary phases, ionic liquid stationary phases SLB-IL 82 and SLB-IL 100 showed comparable resolution, but lower column bleeding with MS detection, resulting in better sensitivity. The selectivity and polarity of the ionic liquid phases are similar to a highly polar biscyanopropyl-silicone phase (e.g. HP-88). In GC×GC, using an apolar polydimethyl siloxane × polar ionic liquid column combination, an excellent group-type separation of fatty acids with different carbon number and number of unsaturations was obtained, providing information that is complementary to GC-MS identification.

In Vivo Pharmacokinetics Study of EH in Rats

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To investigate the pharmacokinetics of EH, a novel recombinant hirudin agent that its bleeding complication risk was markedly reduce by structural modification, after iv administration in rats. Tissue samples were deposited with trichloroacetic acid (TCA-RA) and the radioisotopes tracer method was used to study the pharmacokinetics pattern in rats. Rats were given 2, 4 and 8mg·kg^{-1} EH by *iv.* respectively (n=6). The pharmacokinetic parameters determined by TCA-RA method were as follows: the elimination half-life($t_{1/2}z$)were 6.5±1.7, 6.3±1.7 and 5.3±0.9 h, area under concentration-time curve ($AUC_{last)}$) were 9252±3382, 16543±2064 and 28836±6551 ng·h /mL(1:1.8:3.1), apparent volume (Vz) were 1.5±0.5, 1.7±0.2 and 1.6±0.3 L/kg, clearance (CL) were 0.17±0.05, 0.19±0.03 and 0.22±0.07 L/h/kg, respectively. After *iv* ^{125}I-labeled EH 2mg·kg-1, the rank order of accumulation of ^{125}I-radio labeled in the major organs were as follows: kidney >stomach >bladder >small-intestine >plasma >uterus >lung >hemocyte >liver >ovary >large intestine >spleen >adrenal gland >pancreatic >thymus gland >eyeball >eyeball >heart >skeletal muscle >fatty >brain. The accumulation excretion ratios within 11 days were 65.9±9.6% in urine and only 18.2±6.2% in feces, which was 10.6±2.6% in bile within 48h. Given 2, 4 and 8mg·kg-1 EH by iv respectively, the AUC increased linearly with doses and the systemic clearances were similar among different groups in rats. The results of tissue distribution were as follows: the high concentration in plasma indicated that the macromolecule can not permeate into tissue easily; urine excretion was the major way for its elimination in rat because the EH radioactivity was high in kidney and bladder; the high concentration in stomach and intestine was probably because that EH can secrete into the gastrointestinal cavity through gastric wall and intestinal wall both, and the bile excretion was partly a source of the EH existence in intestine after *iv.* administration.

Development of Liver Malonyl CoA Assay by LC-MS/MS as an Acute In Vivo Efficacy Biomarker

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AMP-activated protein kinase (AMPK) is a key sensor and regulator of intracellular and whole-body glucose and lipid metabolism. AMPK is regulated by phosphorylation and allosteric activators. The objective of the AMPK project is to discover and develop small molecule AMPK allosteric activators for diabetes and dyslipidemia. In order to select compounds for the more resource-intense chronic in vivo efficacy studies, it was critical for us to develop a robust in vivo assay to assess acute efficacy of compound. We first demonstrated that a reduction in liver malonyl CoA level is a downstream readout for in vivo AMPK activity, since liver malonyl CoA formation is catalyzed by acetyl-CoA carboxylase (ACC) which is in turn inhibited by AMPK-mediated ACC phosphorylation. We proposed liver malonyl CoA as a PD biomarker and developed an analytical protocol using LC-ESI-MS/MS method with a throughput of 10 compounds per week. The assay was placed in the critical path of the project, between our cell-based assay and a chronic efficacy study in db mice. The criteria for active compounds was a 20% or greater reduction in liver malonyl CoA level ($p<0.05$) at 4 hrs after p.o. dosing of normal mice at 100 mg/kg. Several active compounds were advanced into the chronic efficacy study in diabetic db mice and showed glucose lowering effects while a few inactive compounds had no efficacy. These results strongly supported the validity and utility of our biomarker assay in guiding compound selection for chronic efficacy studies. The assay played a key role in compound progression to the LSI milestone.

POSTER ABSTRACT

ADME

#37

Determination of Propranolol in Human Plasma by LC-MS

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This paper establish a determination of propranolol in human plasma by LC-MS method, and studied pre-treatment method of protein precipitation and MAS method for plasma samples.This choose MAS method as pre-treatment method. LC-MS detection method for the separation was Venusil ASB C18 (2.1×150mm, 5μm), column temperature was 25°, mobile phase of acetonitrile: 0.01mol/L ammonium acetatesolution (containing 0.1% formic acid) = 60:40, flow rate was 0.2mL/min.Electrospray ionization (ESI); capillary voltage:3.5 kV; ion source temperature:110°; cone voltage :16 V, extraction voltage:3 V, desolvation gas was nitrogen, the temperature was 450°, the flow rate was 600L/h;collision gas flow rate was 0.24mL/min, collision energy was 25V; multiple reaction monitoring (MRM), ion-selective channels were m/z 260.1 ⇢ 183.0.This Methods is reproducible, rapid, accurate, and can be used for the detection of propranolol in human plasma.

Liver Targeting Effect of Vinegar-baked *Radix Bupleuri* on Gentiopicroside in Mice

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In traditional Chinese medicine, the liver targeting enhancing effect is usually come true by co-administration with Vinegar-baked *Radix Bupleuri*, the meridian guide drug of liver. However, the scientific data for this effect are not available. In this paper, the effect of VBRB on the distribution of gentiopicroside (GPS) was investigated.

384 of mice were divided into two large groups according to the GPS dose. In each large group, the mice were further divided into four subgroups, GPS control and GPS co-administered with three different doses of VBRB. Concentrations of GPS in different tissues were determined by HPLC-MS and the target effeciency was evaluated by relative uptake efficiency, relative targeting efficiency, and relative peak concentration.

Compared to the control group, in both GPS dose, VBRB increased the distribution of GPS in liver, and this effect was GPS and VBRB dose depened. High dose of GPS was more sensitive to the effect of VBRB; the effect of VBRB was different when GPS dose different. In HDG, high dose of VBRB had the strongest effect; while in low dosage groups, medium dose had the strongest effect. VBRB affected the distribution by two pathways, first, it increased the uptake of GPS in liver; second, it increased the elimination rate of GPS in other tissues.

In summary, this paper demonstrated that co-administration of gentiopicroside with VBRB is a simple and efficiencient method for liver targeting therapy, and the meridine guide theory of TCM was credible.

Key wards: vinegar-baked *Radix Bupleuri*; gentiopicroside; liver targeting; meridian guide drug; distribution; pharmacokinetics

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POSTER ABSTRACT

ADME

#39

Increased the Sample Loading Capacity For Peptide Analysis by LC-MS/MS using 150 µm ID Packed Tip Columns

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A predominant workflow for qualitative proteomics has been "GeLC-MS," a combination of 1- (or 2-D) gel electrophoresis with reverse-phase nanoflow liquid chromatography mass spectrometry (nLC-MS/MS). The limited protein quantity isolated from a single gel band coupled with column loading capacity maximums necessitate the use of 75 µm ID packed columns for optimal sensitivity.

However, limitations on sample injection volume, gradient and flow characteristics, and excessive delay volume hinder throughput. Novel methods for fractionating complex biological samples with higher loading capacities and more efficient recovery, such as novel solution phase tube-gel fractionation and others, demand a column format which maximizes on the extended dynamic range of these emerging techniques. Packed tip columns with a larger ID (150 to 200 µm) facilitate higher sample loading capacity and enable higher flow rates for improved cycle time while maintaining the optimal sensitivity realized in the nanobore packed tip column format. Using peptide standards, single protein digests and whole yeast digests improvements in cycle time and sample loading capacity utilizing 150 µm ID packed tip columns are demonstrated.

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POSTER ABSTRACT

ADME

#40

Role of Intestinal Bacterial Conversion in Oral Pharmacokinetics of Astragaloside IV, a Marker Compound of Astragali Radix, in the Rat

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Background: Astragaloside IV (AIV) is the most abundant saponin in Astragali Radix, a Chinese herb notable for its adaptogenic properties, and the chemical marker for quality control of Astragali[1]. As a cycloartane-type triterpene glycoside, AIV is likely undergoing stepwise deglycosylation in the gut lumen when administered orally, which might accounted for its low oral bioavailability (<5%) in the rat[2]. In the present study, intestinal bacterial conversion and its role in *in vivo* fate of AIV were examined, for the first time.

Method: AIV (5 uM) was incubated anaerobically with pooled rat intestinal bacteria (RIB) for up to 96h to characterize bacterial conversions. Further, AIV (20 mg/kg) was orally administered to the rat and plasma and feces collected over 48h to determine drug-related components. Samples were qualitatively and quantitatively determined using HPLC/MS/MS. Results: AIV showed a biphasic decline (intact in first 8h followed by a rapid decrease) in RIB and was depleted after 24h. Five metabolites, three via sequential removal of sugar moiety, and two from isomerization and dehydrogenation of the aglycone cycloastragenol, respectively, were identified. Deglycosylation started with C-3 xylose initiated and completed more rapidly than that with C-6 glucose. As a consequence, cycloastragenol exhibited triphasic increase and reached about 50% of the initial AIV after 24h of incubation. Isomerization and deglycosylation were shown to be major and minor pathways of cycloastragenol conversion, respectively, as judged on basis of peak areas of metabolites. When given orally, AIV, cycloastraganol and its isomer were main components detected in rat plasma. In fecal samples, the three components as well as the dehydrogenated product were predominant forms. Conclusion: The significance of intestinal bacterial conversion to systemic exposure and health benefits of AIV was evidenced and warrants further investigational emphasis on metabolites formed in gut lumen. (Supported by the National Basic Research Program of China (973 program, Grant No.:2009CB522707) and the Research Committee of Macau University (Project No.:RG086/09-10S/YR/ICMS))

Keywords: astragaloside IV; intestinal bacteria; hydrolysis; cycloastraganol; isomerization; dehydrogenation

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POSTER ABSTRACT

ADME

#41

Evaluation of Nanospray Voltage and Spray Stability and their Impact on Chromatographic Peak Area

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State-of-the art liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis uses a constant electrospray (ESI) voltage for data acquisition. Modern qualitative and quantitative LC-MS/MS methods depend on highly efficient gradient elution chromatography. The changing chemical composition of mobile phase during gradient elution results in an inherent disconnect with single point ESI voltage optimization. Using a nanospray source equipped with a digitally controlled stage and software for precise and reproducible emitter positioning for data acquisition we investigate the relationship between spray stability and data quality. Repetitive on-column injections at different (fixed) target ESI voltage settings were executed for four separate analytes with a wide range of gradient elution times. Plotting the chromatographic peak area for selected ion currents yields an apparent compound dependant response curve in which a total maximum value is observed. Image capture enabled by the Digital PicoView software program reveals a direct correlation between the observed spray mode, spray stability and chromatographic quality.

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POSTER ABSTRACT

ADME

#42

Renal Organic Anion Transporter 1 (Oat1) as a determinant of Rat Systemic Exposure to Tanshinol of *Salvia miltiorrhiza* (Danshen)

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Tanshinol (TSL), an aqueous component of *Salvia miltiorrhiza* (Danshen), is believed to be responsible for the therapeutic effects of Danshen. Our previous study revealed that approximately 60 percent of dosage after i.v. administration of TSL to rat is eliminated via urine as the unchanged form, and the renal clearance (CL_R) was approximately 6-fold greater than glomerular filtration rate multiplied by unbound fraction in plasma that suggested transporter-mediated active renal secretion of TSL. The purpose of this study is to elucidate the molecular mechanism of the renal secretion using human embryonic kidney 293 cells expressing rat organic anion transporter 1 (Oat1), Oat3, rat organic cation transporter 1 (Oct1) and Oct2 *in vitro*. Furthermore, we also compare systemic exposure to TSL after i.v. administration of TSL with or without probenecid (Oats inhibitor) in rat. As a result, we concluded that TSL serves as a substrate of Oat1, Oat3 but not Oct1 and Oct2; however, Oat1 exhibited higher activity than Oat3. Kinetic study revealed that Oat1-mediated saturable concentration dependent-uptake of TSL was observed in these cells (K_m = 327 μM) and that probenecid inhibited Oat1-mediated uptake of TSL in competitive manner (K_i = 5.3 μM). Pharmacokinetics study were performed after i.v. administration of TSL in absence and presence of probenecid, probenecid decreased CL_R due to impairment of renal secretion, and increased systemic exposure of TSL. We conclude that Oat1 mediated renal secretion of TSL and played a distinct role in determining the systemic exposure to TSL.

Study on Tissue Distribution and Excretion of [^{3}H]Polydipeptide Paclitaxel in Rats

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To study the tissue distribution and excretion of [3H]Polydipeptide Paclitaxel in rats by isotope-labled technology, in order to provide a guidance for the clinic trial. The radioactivity of [3H]Polydipeptide Paclitaxel in tissues, feces, urine and bile of rats were determined by liquid scintillation counter. The tissue distribution experiment showed the radioactivity of [3H]Polydipeptide Paclitaxel was in the order of kidney > blood > liver > spleen > small intestine > stomach > lung > bladder > large intestine > genital gland > thymus > pancreas>brain > suprarenal gland > heart > muscle > gastric > Intestinal contents > fat after 0.5h single i.v. administration (chemical dose 10 mg/kg, radioactive dose 36.3 KBq/g) on rats. The cumulative radioactivity contents excreted in rat urine and feces were 32.4% and 54.7% within 13d. The cumulative radioactivity contents excreted in rat bile was 40.0% within 72h. The total drug excretion rate in feces and urine were 87.1%. Parent drug was mainly distribution in kidney, blood, liver, the rate of excrete of urine and feces was high.

Electrophilicity of Eriocalyxin B: Identification of Glutathione Conjugates in Vitro

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Eriocalyxin B (Eri-B, **1**), an *ent*-kauranoid isolated from medicinal herb *Isodon eriocalyx*, was regarded as the promising candidate for new anticancer agent due to its potent activity. Structure activity relationship studies suggested the predominate role of the electrophilic ∂,ß-unsaturated ketone moieties in ring A and D. In this study, the electrophilicity of Eri-B were evaluated *in vitro* for the first time. When Eri-B was incubated with reduced glutathione in phosphate buffered saline (pH 7.4) at 37°C, three thioether adducts were detected by liquid chromatography-electrospray ionization ion trap mass spectrometry (LC/MS^n) analysis. Two adducts were isolated, and their structures were determined by MS/MS and NMR analysis. The identified product **2** resulted from the Michael addition of glutathione on the conjugated double bond in ring D, while conjugate **3** resulted from the Michael addition of two molecule of glutathione on the conjugated double bond in both ring A and D. Michael addition of glutathione on the conjugated double bond in ring D was found to be significantly faster than in ring A. A dose and time dependent manner was observed for the formation of conjugate **2** from Eri-B. During the first eight hours, the concentration of **2** rises linearly depend on the intial concentration of Eri-B, then decreases slowly accompanied with the formation of conjugate **3**. Further studies will conducted to test the bioactivities of these GSH conjugates.

Pharmacokinetics and Metabolic Property of XQ

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XQ was recently identified as a novel potent inhibitor of acetylcholine esterase, a promising target for Alzheimer's disease. As pharmacokinetic and metabolic properties are likely to be important for efficacy and need to be optimized during drug development, so the aim was to investigate the pharmacokinetics and brain distribution of XQ in mice by i.v., i.g. and s.c. administration. The i.g. dosing absorption was undetectable, but the s.c. bioavailability of XQ was 85% and caco-2 cell experiment indicated that the poor bioavailability was caused by the poor permeability in gastrointestinal, so sub-dermal implants might be a promising formulation of XQ. The half-life of XQ in brain was longer than plasma, which provided material basis for the pharmacological effect. XQ has inhibition on human liver microsome enzyme isoform CYP3A4, but has no significant inhibition on CYP1A2, 2D6, 2C19 and 2C9. As in vitro IC50 for inhibition of acetylcholine esterase was very low, XQ was unlikely to inhibit CYP3A4 in vivo. So the pharmacokinetic and metabolic property of XQ was good and it was promising to develop the sub-dermal implants formulation.

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POSTER ABSTRACT

ADME

#46

High Throughput LDTD-MS/MS Analysis applied for ADME Assays

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LC-MS/MS and now UPLC-MS/MS analysis are the gold standard instrumental method in ADME. However, many reviews point out that it's also the bottleneck of all the ADME process limiting the number of assays to be run and ultimately the number of new drug candidates to be evaluated. The LDTD-MS/MS technology running 1 sample every 10 seconds is proposed to increase the throughput in ADME. The permeability Caco-2/TC7 model was evaluated using 125 new drug candidates. A Pearson correlation coefficient of 0.98 was obtained when the LDTD-MS/MS permeability results were compared to the UPLC-MS/MS results. For cytochrome P450 competitive inhibition assay, the CYP3A4 (testosterone and midazolam) and CYP2D6 (dextromethorphan) were evaluated on 68 and 44 new drug candidates respectively. The results (IC50 values) obtained in LDTD-MS/MS match the one obtained in UPLC-MS/MS as shown by Pearson correlation coefficients higher than 0.91. Finally, the plasma stability assay using both human and rat plasma was evaluated over 12 commercial compounds. The results (half-life time) matched remarkably well between the LDTD-MS/MS and LC-MS/MS. In all cases, the sample preparation was keep the same to run the samples in LDTD-MS/MS. In conclusion, the results demonstrate that the LDTD-MS/MS system generates accurate results leading to the same in house decision making on the new drug candidates, but reducing the analysis time down to 10 seconds per sample.

In vitro Cytochrome P450 Enzymes Inhibition Study of Timosaponin B-II Using Human Liver Microsomes by LC–MS/MS Method

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Timosaponin B-II (TB-II), a furostanol saponin purified from Anemarrhena asphodeloides Bunge-has been reported enhangcing the learning and memory capacities in rats and having an effective action on anti-cerebral ischemia. Inhibition of CYP450 activities by a drug can significantly induce increasing-exposure of co-administrated drugs which are metabolized by the same CYP450 isoenzyme, and resulting in significant adverse events. So *in vitro* determinations of pharmaceutical properties can enhance the effectiveness and safety of TB-II, and promote the reasonable clinical use. We had developed substrate inhibition assays for four of the major CYP450 isoenzymes (diclofenac for CYP2C9, omeprazole for CYP2C19, dextromethorphan for CYP2D6 and testosterone for CYP3A4) in HLM. The four CYP450 inhibitors were ketoconazole (CYP3A4), sulfaphenazole (CYP2C9), ticlopidine (CYP2C19) and quinidine (CYP2D6). Eight concentrations of each the five inhibitors mixture (positive control) and TB-II (2000, 500, 100, 20, 5, 1, 0.2, 0.05μg/ml) duplicatedly was added into the 96 well plate respectively, then the probe substrates were mixed with the HLM and added into the 96 well too. After pre-incubation at 37°C for 5 min, 48μl of NADPH (5mM in 50mM phosphate buffer) was added to each well to give a final volume of 200μl and initiated the reaction. Moreover, a simple, reproducible and sensitive LC-MS/MS method was established for the determination of the analyzing probe substrates, their metabolites and internal standard propranolol simultaneously. The mean IC50 in the positive control was that ketoconazole's was 58.4nM, sulfaphenazole's was 2910.5nM, ticlopidine's was 3144.0nM, quinidine's was 88.0 nM. TB-II had no inhibition on the four CYP450 isoenzymes in the selected concentration. The result can direct the study on clinical drug-drug reactions.

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POSTER ABSTRACT

ADME

#48

Metabolism and Excretion of [^{3}H]Triptolide, A Constituent of Immunosuppressive and Anti-inflammatory Chinese Herb Medicine, in Rat

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Triptolide is the major pharmacological component isolated from the root of *Tripterygium wilfordii* Hook. F., an immunosuppressive and anti-inflammatory Chinese herb medicine. The purpose of this study was to demonstrate the excretion of [^{3}H]triptolide in rats and to investigate its main metabolic pathway. Following single oral administration of [^{3}H]triptolide (0.8 mg·kg^{-1}, 100 μCi·kg^{-1}) to rats without bile duct ligation, total recovery of radioactivity in urine and feces was 88.1% of administered radioactivity. Most of the administered radioactivity was recovered in feces after first 72 h (70.5% compared with 17.5% recovered in urine). Recovery of radioactivity in the bile was 39.2%. On-line radio chromatographic and mass spectrometric analysis revealed extensive metabolism of triptolide. The major components in rat plasma of 15 min included the parent drug and its three mono-hydroxyl metabolites which accounted for 15.4%, 11.3%, 23.0%, and 16.4% of the drug related radioactivity, respectively. One of the major mono-hydroxyl metabolites was identified as 2-hydroxyltriptolide by comparing with the authentic standard. The parent drug accounted for less than 12% of the drug related radioactivity in urine, feces, and bile. Major metabolites in rat urine were characterized as mono-hydroxyl and di-hydroxyl metabolites. In bile and feces, sulfate conjugates of mono-hydroxyltriptolides were identified as the major metabolites for female rats; while mono-hydroxyl and glutathione adduct of triptolide as well as some unidentified metabolites were detected in male rats. In conclusion, triptolide underwent extensive metabolism in rat. Radioactivity was mainly secreted into bile and eliminated in feces. These results intensified the concerning on safety testing of triptolide metabolites.

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POSTER ABSTRACT

Pharmaceutical Sciences

#49

Simultaneous Determination of the pKa and the Solubility of Insoluble Drug Compounds

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Dissociate constant (pKa) is critical parameter in evaluation of drug molecules, affecting drug solubility and species distribution in physiological environment. There are numerous methods are commonly used for determining pKa values of weak acids and bases (potentiometric titrations, spectrophotometry, conductimetry, etc.). However, limited approaches can be used for insoluble pharmaceutical compounds and co-solvent has been often applied. In this study, the solubility method was used to determine the pKa values for insoluble compounds. The solubility was measured at different pH using HPLC or UV UV–visible spectrophotometer. For single pKa , soubilities at two pHs were sufficient, and for two ionization constants, solubilities at three different pH were measured. From measured solubilities, the pKa values can solved from a set of mathematic equations. The total material can be minimum about 5mg. The applicability of the method is demonstrated using ibuprofen, quinine, cimetidine, tetrabenazine and compounds X. The values obtained from this study are very close to the more dependable literature estimates.

POSTER ABSTRACT

ADME

#50

Inhibition of SREBP by a Small Molecule, Betulin, Improves Hyperlipidemia and Insulin Resistance and Reduces Atherosclerotic Plaques

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Sterol regulatory element-binding proteins (SREBPs) are major transcription factors activating the expression of genes involved in biosynthesis of cholesterol, fatty acid and triglyceride. In this study, we identified a small molecule, betulin, that specifically inhibited the maturation of SREBP by inducing interaction of SREBP cleavage activating protein (SCAP) and Insig. Inhibition of SREBP by betulin decreased the biosynthesis of cholesterol and fatty acid. In vivo, betulin ameliorated diet-induced obesity, decreased the lipid contents in serum and tissues, and increased insulin sensitivity. Furthermore, betulin reduced the size and improved the stability of atherosclerotic plaques. Our study demonstrates that inhibition SREBP pathway can be employed as a therapeutic strategy to treat metabolic diseases including type II diabetes and atherosclerosis. Betulin, which is abundant in birch bark, could be a leading compound for development of drugs for hyperlipidemia.

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The PK-PD Study of Oral Insulin Enteric-coated Capsules with Diabetic Wistar Rat Models

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Insulin, as the most commonly used clinical hypoglycemic drugs, has been developed many kinds of administration techniques such as pulmonary, transdermal, nasal, and oral routes. But we have not see any clinical reports about oral insulin practical application in diabetes treatment because of the difficulties such as poor bioavilability and the quality control and stability of preparation. A scientific and validated analytical method suitable for the preliminary PK-PD study of oral insulin enteric-coated capsules was developed. A INSULIN ELISA KIT-Mercodia, Sweden-was used to detect the insulin concentration of the diabetic wistar rat serum. The method has good stability, reproducibility and sensitivity and its concentration range is 3-200 mU/L. The kit has no cross-reaction and small interfering serum matrix. The establishment on the streptozotocin-induced diabetic wistar rat significantly reduced the interference of endogenous insulin. The experiment was performed using four groups of STZ-induced diabetic rat, including subcutaneous administration of 2U/kg, oral administration of 50U/kg and 100U/kg (200U/g and 800U/g insulin content in the two praeparatums, respectively) and control group with empty Capsules. At different times we detected the blood glucose with blood glucose monitoring (OMRON, USA) and the concentration of Insulin by ELISA KIT. The subcutaneous administration group had a rapid absorption and its Tmax was 0.083h. Meanwhile, the level of blood glucose reached to the lowest in the 1-2h. The concentration of the Control and two oral administration groups was lower than the LLOQ and the blood glucose level is higher than 15.0 $mmol \cdot L^{-1}$. The technique of oral insulin enteric-coated capsules need to further improvement.

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POSTER ABSTRACT

ADME

#52

Characterization of In Vitro Glucuronidation of Aloe-emodin, a Main Hydroxyanthraquinone in Rhubarb and Aloe vera

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Background: As a main anthraquinone in Rhubarb and Aloe vera, aloe-emodin (AE) has exhibited an appealing perspective in cancer therapy[1]. An *in vivo* study revealed that AE glucuronides were predominant, yet AE was not detectable in rat plasma when Rhubarb extract was orally administered[2], implying impact of glucuronidation on *in vivo* fates and actions of AE. The present study investigated in vitro glucuronidation of AE for the first time.

Methods: AE (0.5 and 5 μM) was incubated with hepatic microsomal proteins (0.05 mg/mL, human and rat) or recombinant human UGT (0.05 mg/mL, 12 isozymes), respectively, in the presence of UDPGA for 10 min. Metabolites were prepared using a scale-up reaction with rat liver microsomes, isolated using pre-HPLC, and structures identified using LC-MS/MS and NMR (^{1}H, ^{13}C, COSY, DEPT, ROESY) analysis. Quantitative determination was performed on an HPLC-MS/MS system. Results: AE eliminated rapidly in liver microsomes from both species. The average elimination rate of rat was 4 times that of human (1.39 vs 0.45 nmol/min/mg protein). Two metabolites, 8-*O*-glucuronide and 1-*O*-glucuronide, were yielded at different ratios, which was 3:1 with rat yet 2:3 with human at both concentrations. Human UGT1A1, 1A3, 1A7, 1A9 and 1A10 catalyzed conjugation at both positions with different preference. UGT1A9 exhibited the highest activity towards AE glucuronidation at both positions with 1-O-glucuronidation double that of 8-O-glucuronidation. Among isozymes from UGT2 family, only 2B4 and 2B7 showed minor activities at 5 uM.

Conclusions: AE undergoes extensive hepatic glucuronidation in both humans and rats. The glucuronides formed might be related to the health benefits of AE and should be emphasis of further investigation.

Key words: aloe-emodin; glucuronidation; liver microsomes; UGTs;

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POSTER ABSTRACT

ADME

#53

Effective Recording of High Resolution MS/MS Data for Unknown In Vivo Metabolites Through the Use of Mass Defect Filter-Dependent Acquisition

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Drug metabolite identification using high-resolution mass spectrometry (HR-MS) involves acquisitions of HR full-scan MS data for metabolite detection and MS/MS data for structure elucidation. Rapid acquisition of MS/MS spectra of unknown metabolites in complex biological matrices is the key to success, but has always been challenging. Mass defect filter (MDF) has been widely employed for metabolite detection through post-acquisition data processing. The present study was to apply the novel MDF-triggered information-dependent acquisition (IDA) for fast analysis of diclofenac metabolites in rat bile. Online HR MS and MS/MS data were recorded with various IDA workflows using an AB SCIEX TripleTOF™ 5600 system coupled with a Shimadzu HPLC. Altogether 31 diclofenac metabolites, including oxidative metabolites, glucuronides, glutathione adducts and others were identified in rat bile. Many metabolites were identified for the first time. Intensity-IDA recorded MS/MS spectra of only 50% of these metabolites, most of which showed high signal intensities. Dynamic background subtraction (DBS)-IDA greatly enhanced the sensitivity and selectivity, which led to the recording of MS/MS spectra of all but five minor metabolites. This proves that DBS-IDA can be effective in high throughput analysis of in vitro metabolites and major in vivo metabolites. MDF-IDA was able to trigger MS/MS acquisition for all the 31 metabolites in a single injection, some of which displayed low levels of molecular ions and were co-eluted with many endogenous components. These results demonstrate that TripleTOF™ 5600, together with MDF-IDA, is a powerful tool for successful analysis of low levels metabolites in complex matrix.

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#54

A Mechanistic Study on the Unusual Pharmacokinetics of Scutellarin, Using Rats as the Model: Differential Disposition Leads to an Increasing Isoscutellarin/Scutellarin Ratio in the Circulation

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Scutellarin, scutellarein-7-O-glucuronide (S-7-G), is a flavonoid used for treating cardiovascular diseases. After oral administration to humans, instead of S-7-G, scutellarein-6-O-glucuronide (S-6-G) dominated the plasma. The aim of this study was to explain this unusual pharmacokinetic characteristics by studying the absorption and disposition of S-7-G, using rats as the model. Based on the observed rapid hydrolysis of S-7-G in rat intestinal content and enhanced Caco-2 cell permeability of its hydrolysis product, it was naturally inferred that S-7-G was absorbed as its aglycone (scutellarein). According to the metabolism of aglycone in rat intestinal S9 and intra-intestinal infusion experiment with either aglycone or S-7-G, aglycone was demonstrated to be conjugated extensively in intestine yielding S-7-G and S-6-G at a ratio of 20:1, which subsequently went into mesenteric plasma at a ratio of 15:1. However, in contrast to absorption, the S-7-G/S-6-G ratio in systemic circulation was merely 1:1, possibly attributed to the differential pre-systemic clearance in body. In the excretion study in rats, S-7-G was found to be excreted in a greater amount than S-6-G (5.5:1) into bile, which was the major excretion route. Furthermore, S-7-G had a significantly higher glucuronidation rate than S-6-G in RLMs, forming scutellarein-6,7-O-diglucuronide, the most abundant metabolite in rats. Conclusively, after oral administration, S-7-G was hydrolyzed into aglycone, which was then re-conjugated in intestine. S-7-G and S-6-G were absorbed into blood at ratio 15:1, but appeared in systemic circulation at ratio 1:1 due to a greater bile excretion and higher glucuronidation rate of S-7-G.

Preparation and Characterization of Self Nano-Emulsifying Drug Delivery System

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Over 65% of commercially available drugs are formulated for oral administration. However, one of the major factors limiting the effectiveness of orally administered drugs is poor absorption from the gastrointestinal tract or extensive presystemic clearance. For poorly water-soluble drugs, slow dissolution rate in the primarily aqueous contents of the gastrointestinal tract presents a significant barrier to absorption. Recently most interest have been given to self- nanoemulsifying drug delivery system (SNEDDS), that are administered as an isotropic preconcentrate and form submicron size emulsions upon dispersion in the GI tract.

To enhance oral absorption of poorly water-soluble drug, SNEDDS was formulated. SNEDDS is a mixture of lipid, surfactant, and cosurfactant, which are emulsified in aqueous medium under gentle digestive motility in the gastrointestinal tract.

Solubility of drug was determined in various vehicles. Pseudo-ternary phase diagrams was constructed to identify the efficient self-microemulsification region. SNEDDS was prepared and characterized for particle size, zeta-potential, and in vitro dissolution rate. The mean particle size of SNEDDS formulation after dispersion was about 160.6 nm. The dissolution rate from SNEDDS was significantly higher than the conventional tablet. Thus, it was concluded that SNEDDS provide an effective approach for the delivery of hydrophobic compounds by oral route.

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POSTER ABSTRACT

Pharmaceutical Sciences

#56

An Dried Blood Spot Approach to Overcome Stability Challenge for Kol43 in Mouse Blood

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Dried blood spot approach has demonstrated significant advantage in replacement, reduction and refinement. An investigation was carried out to evaluate the potential advantage of DBS in overcoming stability challenge for test compounds in biological matrices. Ko143 is widely accepted as a potent breast cancer resistance protein (BCRP) inhibitor and has been used to identify potential BCRP substrates in both *in vitro* and *in vivo* experiments. Rapid conversion of Ko143 into its carboxylate product by esterase has been previously noted, hence, it remains challenge to provide accurate quantification of Ko143 and its metabolite in biological matrices. Chemical treated Whatman™ FTA DMPK-B cards were utilized to evaluate the stability of Ko143 during sample collection and preparation. The responses of Ko143 and its metabolite were monitored by UPLC-MS/MS. Our data suggested less than 1% Ko143 remaining after 8hrs in mouse whole blood whilst sample prepared on DBS card showed less than 2.4% Ko143 was converted into its metabolite after 8hrs under ambient condition, confirming the advantage of DBS card in minimizing esterase activity from fresh mouse whole blood. The extraction recovery from DBS cards was found at approximate 86%. Good accuracy and precision of quantification results for both Ko143 and its metabolite in mouse whole blood suggested the predominance of DBS technique to overcome stability challenge and provide reliable quantification for biological samples.

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#57

Oral Pharmacokinetics of Baicalin, Wogonoside, Oroxylin-A-glucuronide and their Aglycones from Scutellariae Radix in the Rat: Intestinal Bacterial Conversions, Transepithelial Permeability, and Hepatic Isomerization

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Background: Scutellariae Radix (SR) has been extensively prescribed due to notable medicinal benefits. Flavonoids, including glucuronides baicalin, wogonoside, oroxylin-A-glucuronide and their aglycones baicalein, wogonin and oroxylin-A, were considered as the main active components of the herb. So far, in vivo fates of all six flavonoids in SR extract and the determinant factors were not yet fully characterized.

Methods: Kinetics of six flavonoids in plasma and their excretion profiles in urine and feces within 48h after oral administration of SR extract (800 mg/kg) to rats were simultaneously determined using LC-MS/MS. Glucuronide isomers formed in vivo were also semi-quantitated. Intestinal bacterial conversion and transepithelial permeability of the flavonoids in the extract and isomerization of the glucuronides were characterized in vitro. Results: Both glucuronides and aglycones stayed in plasma over 48h with AUCs of glucuronides 10-100 times that of respective aglycones. AUPs (area under peak area ratio-time curve) of baicalin isomers were ~12 times that of baicalin yet AUP of wogonoside isomer only one seventh of wogonoside. Glucuronides were the main forms in urine, whereas aglycones predominated in feces, throughout the experiment. Glucuronides were rapidly converted by rat intestinal bacteria (RIB) to respective aglycones. Both glucuronides and aglycones traversed Caco-2 cell monolayers via passive diffusion with the latter bearing >10 times higher permeability. There were remarkable (baicalein), minor (wogonin) and no (oroxylin-A) isomerization in rat liver microsomes while isomerization is absent in RIB.

Conclusions: Oral kinetics of six flavonoid glucuronides and aglycones in SR were simultaneously obtained, for the first time. Intestinal bacterial conversion, transepithelial permeation and hepatic isomerization are determinant factors for their systemic exposures. (supported by the National Basic Research Program of China (973 program, Grant No.:2009CB522707 and the Research Committee of Macau University (Project No.:RG086/09-10S/YR/ICMS).

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The Preclinical Pharmacokinetics Study of MS-001

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A sensitive, specific HPLC-MS2 method was established and validated for determination of MS-001 in preclinical pharmacokinetics study. The pretreatment of plasma sample and tissue sample were precipitation of protein using methanol. Rabeprazole was used as an internal standard for the quantitation. Following a single oral administration with different dose of MS-001 to Wistar Rats (6, 30 and 150mg/kg), Beagle Dogs (1, 3 and 9 mg/kg) ,the blood samples were collected at different time after dosing. Wistar Rats were serially euthanized by spondylopathy luxation at 15min, 4h, 12h after p.o. administration (30mg/kg) to study the tissue distribution of MS-001. Tissues were taken out and made into homogenate preparation with 2 volume water. Bile, urine and feces of rats were collected after p.o. administration (30mg/kg), then the concentration of MS-001 and metabolite were determined by HPLC-MS2 method. Plasma protein binding was determined by ultrafiltration with final concentration of 50, 1000, 8000ng/ml. Good liner was obtained within the range of 2-1000ng/ml, The precision of intra-assay and inter-assay was evaluated by analysis of variance with the result of 5.18~13.65% and 5.5~10.19%, respectively. The matrix effects and the dilution integrity of the method wasn't found. Following a single oral administration with different dose of MS-001 to Wistar Rats (6, 30 and 150mg/kg), Beagle Dogs (1, 3 and 9 mg/kg),The ratio of the area under concentration-time curve (AUC) in rats is (1:28.8:192.4) and in Beagle Dog is (1:4.7:21.8) across the three dose levels. The absolute bioavailability of MS-001 was 84.39±26.1% in rats and 64.4±22.3% in Beagle Dog. The concentration of every tissue and body fluid were all high at 15 min , remarkably decrease at 4h, part of tissues were under 10ng/ml after 12h. It was shown that about 2.20±0.90% of dosage was excreted in urine 3.51±1.05% in feces and 1.12±0.29% in bile within 96h.The unchanged compound and metabolites were observed less than 4% in the three excretion pathway. The result showed that MS-001 might metabolize extensively. The plasma protein binding ratio of MS-001 in rat, dog and human were all around 98%. The present investigation showed that this method is simple, accurate, sensitive reproducible and it was successfully applied to pharmacokinetic study of MS-001, and it provides crucial information for its clinical research.

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High Sensitivity Protein Quantitation Using a Triple Quadrupole with a Dual Ion Funnel

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Assays that are both specific and quantitative for target proteins are critical for preclinical validation of putative biomarkers. Such assays are typically multiplexed, multiple reaction monitoring (MRM) analyses which can provide the high-throughput required. Sensitivity is a key requirement for such assays as protein biomarker concentrations may be quite low in commonly used biofluids such as serum and plasma. Improving the sensitivity of LC/MS can be achieved by using nanoflow LC, and by enhancing the sampling and transmission of ions in the mass spectrometer. This study demonstrates the 5-10x sensitivity gain achieved for peptides using a triple quadrupole mass spectrometer modified with a dual ion funnel. The sensitivity achieved using a microfluidic-based nanoflow LC system compared to a standard LC system will be discussed.

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Transmission Mode Direct Analysis in Real Time (DART): Enabling High Throughput Open Air Desorption Ionization for Bioanalysis and Beyond

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A fourth generation DART ionization source (DART-SVP) fitted with a two dimensional 96-well plate footprint X-Z transmission experiment module permits the analysis of 96 samples in just under 16 minutes. The transmission module is designed to hold a woven wire cloth sampling surface, and potentially blood spotting paper vertically in front of the DART ionization source. The sample surface is then moved systematically within the DART ionization region.

The determination of small molecules directly from in-vitro assays including plasma protein binding and microsomal metabolism studies has been improved by enabling the X-Z transmission stage. Mounting the sample plate vertically allows the desorption gas to flow directly onto the sample and then through the porous sample surface. One major improvement in this experimental set-up over previous configurations is that the samples can be placed significantly closer to the inlet of the mass spectrometer resulting in an increase in analyte sensitivity, improved reproducibility and overall throughput with respect to our previous work in this area. The previous DART studies conducted in the bioanalytical lab have always been performed off of solid glass surfaces, which set the benchmark for DART-MS/MS analysis directly from plasma. Moving forward, analysis of drug compounds directly out of whole blood off of blood spotting papers will be one of the main focuses for this new technology. In addition to the benefits noted for the X-Z sampling stage in the bioanalytical laboratory, this set-up has also enabled us to tune for compound dependent MS parameters using the DART source.